Evaluation of Relationship Between Serum Neopterin, Cystatin C and Coronary Heart Disease

Serum Neopterin, Sistatin C ve Koroner Kalp Hastalığı Arasındaki İlişkinin Değerlendirilmesi

Neopterin, Cystatin C and Gensini Score

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Özet

Amaç: İmmun yanıtın düzenlenmesinde anahtar bir molekül olan neopterinin aterom plaklarda aktif makrofajlardan salınmakta olup kardiyovasküler risk sınıflamasında faydalı olduğu bildirilmiştir. Bu çalışmanın amacı, koroner arter hastalarında serum sistatin C ve neopterin düzeylerini araştırmak ve bu belirteçler ile diğer kardiyovasküler risk faktörleri arasındaki ilişkiyi incelemektir. Gereç ve Yöntem: Koroner arter hasta ve kontrol grupları sırasıyla 75 (61 erkek, 14 kadın, ortalama yaş 60.5±10) ve 50 (25 erkek, 25 kadın, ortalama yaş 56.9±10) kişiden oluşmaktadır. Bulgular: Koroner arter hasta grubunda serum neopterin konsantrasyonları kontrol grubuna göre yüksekti. Neopterin ile yüksek dansiteli lipoprotein kolesterol arasında negative korelasyon vardı. Aynı zamanda Gensini skoru ile sistatin C ve neopterin arasında pozitif korelasyon mevcuttu. Tartışma: Koroner arter hastalığının şiddetini saptamada serum sistatin C ve neopterin konsantrasyonları kullanışlı belirteçler olabilir. Bu çalışmanın sonuçlarına gore neopterin, sistatin C'ye kıyasla kardiyovasküler hastalıkların daha güçlü bir göstergesi olarak görünmektedir. Gensini skoru ile biyokimyasal belirteçler arasında bulunan bu korelasyon bize hastalık aktivitesinin değerlendirilmesinde bu belirteçlerin kullanılabileceğini göstermektedir.

Anahtar Kelimeler

Neopterin; Sistatin C; Ateroskleroz; Koroner Arter Hastalığı; Gensini Skoru

Abstract

Aim: It has been reported that neopterin, a key molecule for immun response organization, is released from active macrophages of atheromatous plaque and useful for cardiovascular risk stratification. The aim of this study was to investigate serum cystatin C and neopterin levels in patients with coronary artery disease and examine the relationship between these markers and cardiovascular risk factors. Material and Method: Coronary artery disease group and control group consisted of 75 subjects (61 males, 14 females, mean age 60.5±10) and 50 subjects (25 males, 25 females, mean age 56.9±10), respectively. Results: Serum neopterin concentrations were higher in coronary artery disease group compared to control group. A negative correlation was established between neopterin and high density lipoprotein cholesterol. Also, there was a positive correlation between Gensini score and cystatin C and neopterin. Discussion: Serum cystatin C and neopterin concentrations might be useful markers for determining the coronary artery disease's severity. According to this study, neopterin seems to be a stronger indicator of cardiovascular diseases compared to cystatin C. The positive correlation between Gensini score and the biochemical markers offers us a potential use of these markers in evaluating the disease's severity.

Keywords

Neopterin; Cystatin C; Atherosclerosis; Coronary Artery Disease; Gensini Score

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Introduction

Cardiovascular diseases (CVD) are one of the leading cause of death for individuals from different ethnic groups. Atherosclerosis plays a substantial role by narrowing the coronary arteries [1]. The important role of lipids in the development of atherosclerosis was described by Ross in 1986 [2]. In addition to hyperlipidemia, the role of inflammation in atherosclerotic plaque generation and progression is yet incontestable. [3]. Hence, understanding the effect of inflammation in atheromatous plaque growth and rupture captured attention to consider about new inflammatory markers and select the best assay to define CVDs at risk [4].

Neopterin is generated by monocytes and macrophages in response to stimulation by a cytokine originating mainly from activated T-helper type-1 lymphocytes, interferon-γ and natural killer cells. Hydrolysis and oxidation of 7,8- dihydroneopterin triphosphate is produced the neopterin. It originates from the conversion of guanosine triphosphate by the action of guanosine triphosphate– cyclohydrolase I. The amount of released neopterin is strongly related to the release of reactive oxygen radicals by cells. High serum levels of neopterin in atherosclerotic diseases indicated that immunological mechanisms might be responsible for atherosclerosis development and complications [5]. It has been reported that neopterin, a key molecule for immun response organization is released from active macrophages of atheromatous plaque and useful for cardiovascular risk stratification [6].

Cystatin C is a low molecular weight protein produced in all nucleated cells at a constant rate and excreted into bloodstream. Due to low molecular weight and cationic properties, it is freely filtered through glomerulus, reabsorbed and completely metabolized by proximal tubule cells. For this reason, cystatin C is an ideal biomarker for estimating glomerular filtration rate [7]. Recently, it is considered as a stronger predictor than creatinine for glomerular filtration rate [8]. The lysosomal cysteine cathepsins have been shown to be implicated in the development and progression of atherosclerosis. Cystatin C is a major endogenous inhibitor of cysteine cathepsins with the highest inhibiting activity to cathepsin L and S. Cystatin C is decreased in atherosclerosis site [9]. It has been proposed that elevated cystatin C levels correlated with inflammation and atherosclerosis. Cystatin C is independently associated with cardiovascular disease after adjustment for major cardiovascular risk factors [10].

The aim of this study was to investigate serum cystatin C and neopterin levels in patients with coronary artery disease and examine the relationship between these markers and cardiovascular risk factors.

Material and Method

Study Design

This study was conducted in biochemistry department and cardiology clinic of Ataturk Education and Training Hospital, İzmir. 125 subjects who had an angiogram in Cardiology Department were recruited to this study. Smoking status, chronic diseases, electrocardiograms and ejection fractions (EF) were recorded. Elective coronary angiography were performed by cardiologists. The stenosis of the vascular lumen of about 70% or more was diagnosed as critical coronary stenosis. The coronary angiographies were interpreted by the "coronary artery disease severity score". In this scoring, the previously defined Gensini score was used. The coronary arterial tree was investigated in a segmental fashion. According to the functional importance, the multiplication factor was 5 and 0.5 for the main coronary artery and the distal segments, respectively and it was multiplied with the luminal diameter scores (0, 1, 2, 4, 8, 16, and 32). At the end a total Gensini score (14) reflecting the severity of the coronary artery disease was obtained as a numerical value. Subjects with systolic blood pressure (SBP) ≥ 140 mmHg, diastolic blood pressure (DBP) ≥ 90 mmHg and/or those using antihypertensive medication were classified as hypertensive. Hyperlipidemia was diagnosed in subjects when the 12 hours blood total cholesterol (TC) was ≥ 220 mg/dl, low density lipoprotein (LDL) was ≥ 100 mg/dl and triglyceride (TG) was ≥ 150 mg/dl and/or in subjects taking antihyperlipidemic drugs. Diabetes mellitus in subjects was defined as glucose levels ≥ 126 mg/dl, hemoglobin (Hb) A1c ≥ 6.5 % or those who were being treated with oral antidiabetic drugs or insulin. Coronary artery disease group (CAD) comprised of 75 subjects (61 males, 14 females, mean age ±SD; 60.5±10) after Gensini score classification (14). Control group comprised of 50 subjects (25 males, 25 females, mean age ± SD; 56.9±10). The patients who participated the study were using various drug therapies at the time of the application and there was no diagnosis of coronary artery disease in any of these patients: 14 (14.6 %) patients with amlodipine, 61 (81.3%) with acetylsalicylic acid, 4 (5.3%) with atenolol, 2 (2.6%) with benidipine, 1 (1.3%) with gliclazide, 8 (10.6%) with metformin, 7 (9.3%) with metoprolol, 1 (1.3%) with nifedipine, 1 (1.3%) with perindopril, 2 (2.6%) with pioglitazone, 1 (1.3%) with zofenopril and 16 (21.3%) with hydrochlorothiazide. The subjects with no diagnosis of coronary artery disease were selected and consisted of control group. Individuals who were suffering from acute illness other than acute coronary syndromes or who had chronic non-cardiac diseases or malignancy within the last five years were excluded. The study was approved by the local ethics committee (Ethical approval date and number :29/01/2009-533). Informed written consent was obtained from all participants. Serum samples were collected from all participiants, seperated by centrifugation at 4000 rpm for 5 minutes and stored at -80 0C until analysis. Serum samples were divided into two aliquots and immediately kept away from light and frozen in dark at -80 0C until neopterin analysis.

Biochemical Measurements

Glucose, Total cholesterol, high density lipoprotein cholesterol (HDL-cholesterol), low density lipoprotein cholesterol (LDL-cholesterol), triglyceride, C-reactive protein (CRP), creatinine, Blood urea nitrogen (BUN) were analyzed with commercial kits on Abbott Architect c16000 autoanalyzer. Also, cystatin C was analyzed by particle enhanced turbidimetric immunoassay (PETIA) kit (Cat No: 1P93-01) on Abbott Architect c16000 autoanalyzer. The intra-assay coefficent of variation was <5% for cystatin C measurement. Neopterin serum concentrations were determined using an enzyme linked immunosorbentassay (Elisa Kit Ref No IB29125, IBL, Germany). The detection limit was 0.7 nmol/L and the intra-assay coefficient of variation was 5.5% in

the range of 7.7 nmol/L and 3.6% in the range of 27 nmol/L for neopterin measurements.

Statistical Analysis

Statistical analysis was performed using the SPSS v10.0 Statistical Package for Social Sciences (SPSS Inc, USA). The two groups were compared with an unpaired Student's t test or with a Mann–Whitney U test according to normality. Categorical variables were compared using the x2 test or Fisher exact test. Analyzing by One-Sample kolmogorov-Smirnov test, cystatin C, neopterin, glucose, triglyceride, creatinine and CRP were found to have non-homogeneous distrubition. Sperman's correlation anaylsis was used for cystatin C, neopterin, glucose, triglyceride, creatinine and CRP and Pearson correlation analysis was performed for LDL, total cholesterol, HDL and BUN. p=0.05 were considered significant. Lineer regression analysis was performed by adjusting with age, sex, presence of diabetes, hypertension and smoking, EF, EKG and eGFR.

Results

There was a significant difference in age or male/female ratio between patients and controls (p<0.001). Smokers were more common in CAD patients (54%) than controls (48%), with a non-significant difference (p=0.472). Demographic data obtained from CAD patients and controls are presented in Table 1.

HDL-C levels were significantly decreased between the patients and the control group [(p=<0.001)], whereas total cholesterol levels were not statistically significant [(p=0.075)].Laboratory values of both groups were presented on Table 2.

Neopterin and Cystatin C concentrations were higher in CAD group compared to control group (p<0.001 and p=0.081, respectively) (Figure 1).

There was a positive correlation between cystatin C and age, CRP (r=0.306, p=0.001; r=0.257, p=0.004 respectively). A negative correlation was established between neopterin and HDL-C (r=-0.225, p=0.012). There was a positive correlation between Gensini score and cystatin C and neopterin (r=0.206, p=0.021 and r=0.403, p<0.001; respectively) (Figure 2).

In multivariate lineer regression analysis, cystatin C was found to change significantly with age (p=0.022) and remain constant after adjustment with sex, presence of diabetes, hypertension and smoking, EF, EKG and eGFR (p>0.05). Neopterin values did not differ after adjustment with age, sex, presence of diabetes, hypertension and smoking, EF, EKG and eGFR (p>0.05).

Table 1. Demografic variables of both groups.

	Control group (n=50)	CAD group (n=75)	p
Age (Years)	56.9±10	60.5±10	0.059
Sex (M/F)	25/25	61/14	<0.001*
Hypertension (%)	48	56	0.039*
Hyperlipidemia (%)	48	54	0.472
Diabetes (%)	20	16	0.635
Smoking Habit (%)	48	54	0.472
ECG positivity (%)	14	22	0.254

There were no significant differences in age or male/female ratio between patients and controls (p>0.05). Smokers were more common in CAD patients (54%) than controls (48%), but it did not show a significant difference (p=0.472). Data were expressed as mean ± standard deviation. *= p<0.05 is considered as statistically significant
Abbreviations: ECG, electrocardiogram; M,male and F,Female.

Table 2. Laboratory values of control and CAD groups.

	Control group (n=50)	CAD group (n=75)	p
CRP (mg/dL)	0.46±0.93	0.81±2.42	0.188
Glucose (mg/dL)	106±20	106±30	0.572
Trigliserid (mg/dL)	131±72	163±95	0.039*
HDL-Cholesterol (mg/dL)	44±12	37±10	<0.001*
LDL-Cholesterol (mg/dL)	115±32	130±40	0.026*
Total Cholesterol (mg/dL)	185±38	200±49	0.075
BUN (mg/dL)	15.2±3.5	16.6±4.8	0.143
Creatinine (mg/dL)	0.82±0.14	0.92±0.20	0.001*
EF (%)	61±6	58±8	0.004*
eGFR	86.9±18.7	95.5±24.2	0.226
Gensini Score	1.03±1.51	40.2±31.9	<0.001*

HDL-cholesterol (HDL-C) levels were significantly decreased between the patients and the control group [(p=<0.001)], whereas total cholesterol levels were not statistically significant [(p=0.075)]. Data were expressed as mean ± standard deviation. *= p<0.05 is considered as statistically significant
Abbreviations: CRP,C-reactive protein; BUN,Blood urea nitrogen, EF, ejection fraction; eGFR, estimated glomerular filtration rate.

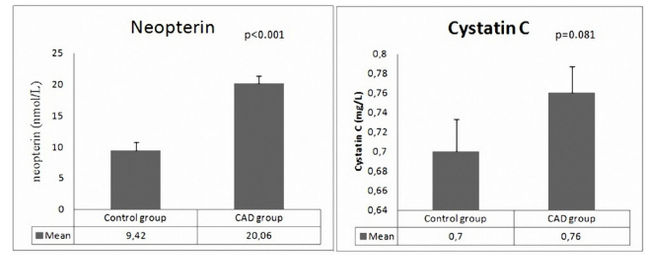

Figure 1. Comparision of Neopterin and Cystatin C concentrations of both groups. Neopterin and Cystatin C concentrations were higher in CAD group compared to control group. (9.42±1.26, 20.06±2.85 ; 0.70±0.23, 0.76±0.31, respectively) CAD=Coronary artery disease. Values were expressed as mean±SD

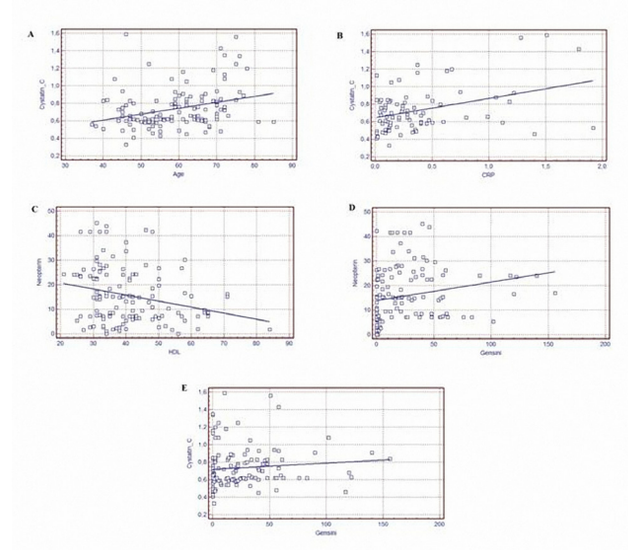

Figure 2. Graphics of significant correlations in CHF patients. (A) Cystatin C and Age (r=0.306, p=0.001); (B) Cystatin C and CRP (r=0.257, p=0.004); C)Neopterin and HDL (r=-0.225, p=0.012); (D) Neopterin and gensini (r=0.403, p<0.001); (E) Cystatin C and Gensini (r=0.206, p=0.021)

Discussion

This study demonstrated a significant increase in neopterin concentrations in subjects with CAD compared to healthy subjects. There was a positive correlation between cystatin C and age, CRP. A negative correlation was established between ne-

opterin and HDL-C. There was a positive correlation between Gensini score and cystatin C and neopterin. Neopterin, pteridine derived molecule, is produced as a response to γ-interferon released from activated macrophages. Neopterin is considered as a useful prognostic marker for classification of coronary heart disease patients. Neopterin has predicted the progression of coronary artery disease. A positive correlation has been reported between serum neopterin levels and carotid atherosclerosis [11]. Schumacher et al found that serum neopterin concentrations are higher in patients with acute myocard infarctus (AMI) [12]. Higher neopterin concentrations were presented in coronary heart disease. Ozbek C et al performed a study with 40 stable angina pectoris patients and found a positive correlation between serum neopterin and Gensini scores as in this study [13]. High levels of neopterin may be due to inflammation, a compound of endothelial dysfunction. Besides the predictive role for clinical prognosis, neopterin might also have a pathogenic property. In such a way that it is released as a response to γ-interferon from activated macrophages that have a key role in deterioration of atheroma plaques. Neopterin might have an indirect interaction with intracellular signal pathways that is affected by oxidative stress and may cause overproduction of nuclear factor κB translocation in atherogenesis and expression of proinflammatory genes, adhesion molecules, tissue factors and several proteins in atheroma plaque related to rupture and disease prognosis.

An interesting observation in this study was a negative correlation between neopterin and HDL-C. HDLs perform functions including several immunological activities. The atheroprotective functions of HDL that have more recently attracted attention among other actions include, its anti-apoptotic, anti-thrombotic and anti-infectious functions. HDL can directly inhibit oxidation of LDL; or other targets containing phospholipids. In addition, inhibition of oxidative events and oxidative stress in vivo may be achieved indirectly via other functions of HDL, such as induction of cholesterol efflux and, in general, via 'anti-inflammatory' functions of HDL. The antioxidative activities of HDL might equally become impaired in the presence of inflammation due to the altered enzymatic activities. Normal functional HDL has high levels of anti-oxidants and active anti-oxidant proteins and enzymes with high anti-oxidant potential and has anti-inflammatory activity. As part of the acute-phase response, activities of HDL-associated enzymes [14]. This interesting obeservation may be due to anti-aterogenic effect of HDL-C. Inflammation is a chronic process. Although HDL-C has protective effects for atherosclerosis development, this may not break down the inflammatory period.

Clinical studies have showed that the risk of cardiovascular disease, myocardial infarctus, stroke, angina pectoris increased with high serum cystatin C concentrations [15,16]. Serum cystatin C concentrations of CAD group was higher than control group but this association was not statistically significant. Serum cystatin C is affected by several conditions as age, inflammation. The mean age in both group was different. This may alter the cystatin C results. The significance was 0.081. Studies with larger study participiants might give an importance value ($p<0.05$). John Beilby et al compared the predictive role of serum cystatin C, serum creatinine and creatinine clerance in cardiovascular outcomes. After a 10 years follow-up period, these markers were found statistically relevant with mortality and morbidity. Cystatin C was not superior than other parameters for predicting the risk [17]. In a study with type 2 diabetes mellitus patients, Eun Hee Kim reported no association between serum cystatin C and cardiovascular diseases. They reported that carotid atherosclerosis, not serum cystatin C concentrations, is associated with microalbuminuria [18].

Conclusion

Both serum neopterin and cystatin C correlate with Gensini score and they might be useful indicators of disease severity. Neopterin seems to be a stronger indicator of cardiovascular diseases compared to cystatin C. Data are needed to better define the relationship between cystatin C, neopterin and coronary artery disease, which may clarify its relationship with atherosclerosis and cardiovascular mortality and elucidate new mechanisms which may lead to development of novel therapies.

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Competing interests

The authors declare that they have no competing interests.

References

1. Murray CJ,Lopez AD. Global mortality disability and the contribution of risk factors: Global Burden of Disease study. Lancet 1997;349(9063):1436-42.
2. Ross R. The pathogenesis of atherosclerosis. The New England Journal of Medicine 1986;314(8):488-500.
3. W van Lammeren G, L Moll F, Borst GJ, de Kleijn DP, P M de Vries JP, Pasterkamp G. Atherosclerotic plaque biomarkers: beyond the horizon of the vulnerable plaque. Curr Cardiol Rev 2011;7(1):22-7.
4. Pearson TA, Mensah GA, Alexander RW, Anderson JL, Cannon RO, Criqui M et al. Markers of inflammation and cardiovascular disease: application to clinical and public health practice. A statement for healthcare professionals from the Centers for Disease Control and Prevention and the American Heart Association. Circulation 2003;107(3):499-511
5. Vera R, Anita S, Uldis K, Andrejs E, Matthias J, Karlis T et al. Association between increased serum neopterin and homocysteine concentrations as well as pyridoxal-5-phosphate deficiency in patients with coronary heart disease. Pteridines 2001;12(3):130–4.
6. Ray KK, Morrow DA, Sabatine MS, Shui A, Rifai N, Cannon CP et al. Long-term prognostic value of neopterin: a novel marker of monocyte activation in patients with acute coronary syndrome. Circulation 2007;115(24):3071-8.
7. David J N. Cystatin C.Annals of Clinical Biochemistry 2002;39(2):89-104.
8. Dharnidharka VR. Serum cystatin C in superior to serum creatinine as a marker of kidney function: a meta-analysis. Am J Kidney Dis 2002;40(2):221-6.
9. Gu FF, Lu SZ, Chen YD, Zhou YJ, Song XT, Jin ZN et al. Relationship between plasma cathepsin S and cystatin C levels and coronary plaque morphology of mild to moderate lesions: an in vivo study using intravascular ultrasound. Chin Med J (Engl) 2009;122(23):2820-6.
10. Arinell K, Christensen K, Blanc S, Anders L, Ole F. Effect of prolonged standardized bed rest on cystatin C and other markers of cardiovascular risk. BMC Physiol 2011;9:11-17.
11. Weiss G, Willeit J, Kiechl S, Fuchs D, Jarosch E, Oberhollenzer F et al. Increased concentrations of neopterin in carotid atherosclerosis. Atherosclerosis 1994;106(2):263-71.
12. Schumacher M, Halwachs G, Tatzber F, Fruhwald FM, Zweiker R, Watzinger N et al. Increased neopterin in patients with chronic and acute coronary syndromes. J Am Coll Cardiol 1997;30(3):703-7.
13. Ozbek C, Baran I, Tütüncü A, Kuştarcı T, Karaağaç K, Ozbek A. Modest correlation between serum neopterin levels and Gensini scores in a cohort of patients undergoing coronary angiography. Ir J Med Sci 2014;183(2):297-301.
14. Eren E, Yilmaz N, Aydin O. High Density Lipoprotein and it's Dysfunction. The Open Biochemistry Journal 2012;6:78-93.
15. Myers G, Robert HM, Christenson RH, Mary C, Ballantyne CM, Cooper GR et al. National Academy of Clinical Biochemistry Laboratory Medicine Practice Guidelines: Emerging Biomarkers for Primary Prevention of Cardiovascular Disease. Clinical Chemistry 2009;55(2):378–84.
16. Parikh NI, Hwang SJ, Yang Q, Larson MG, Guo CY, Robins SJ et al. Clinical cor-

relates and heritability of cystatin C (from the Framingham Offspring Study). Am J Cardiol 2008;102(9):1194-8.
17. Beilby J, Divitini ML, Knuiman MW, Rossi E, Hung J. Comparison of Cystatin C and Creatinine as Predictors of Cardiovascular Events in a Community-Based Elderly Population. Clinical Chemistry 2010;56(5):799-804.
18. Eun HK, Ji HY, Sang AL, Eui YK, Won GK, Seung HL et al. Lack of Association between Serum Cystatin C Levels and Coronary Artery Disease in Diabetic Patients. Korean Diabetes J 2010; 34(2):95–100.

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